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VOL. 29 SEPTEMBER, 1951 NUMBER 9

A CONTINUOUS MOTION CAMERA FOR MULTIPLE EXPOSURE OF 35 mm. FILM[1]

BY E. L. R. WEBB

Abstract

This is a brief description of a special continuous motion camera. It makes possible the economical recording, at 4 in. per second, of transient signals by the method of photographing the deflection modulated spot of a cathode ray tube. The film is passed through the camera 20 times, writing one wavy line each trip, and thus recording 2000 ft. of information on a standard 100 ft. roll of film. Various features of automatic operation are included.

The photography of a moving spot of light on a moving film extends the spectrum of transient signals beyond that possible with a moving pen or other mechanical systems. The upper limit of a typical inking recorder is 100 c.p.s. with a chart speed of 4 in. per second. Because of higher resolution it is possible to record up to several hundred cycles per second on film moving at a similar speed. The signal is applied to the horizontal deflection plates of a cathode ray tube upon whose screen the camera is focused.

At a speed of 4 in. per second a 100 ft. roll of film lasts only five minutes, so for reasons of convenience and economy it was proposed to pass the film through the camera many times. As a preliminary step an existing camera was modified to increase the film speed and to provide for reversal of the direction of travel of the film. Three motors were used and all switching was accompanied by means of relays—including a stepping relay that functioned both to shift the spot and also to keep count of the number of trips. Reversing was accomplished by a latching type relay operated by limit switch impulses, the limit switches being actuated by a nut traveling on a lead screw cut on an extension of the drum spindle. The system worked but was limited by the stepping relay to eight passes, and it required considerable care in the setting up of the controls after loading a film. Also it was possible for the stepping and reversing relays to get out of step during power outages, and for these reasons further development work was undertaken.

As a result the camera mechanism and its operation are now quite simple and nearly foolproof. All but two relays have been eliminated, and one of

[1] *Manuscript received March 15, 1951.*
Contribution from Radiophysics Section, Electrical Engineering and Radio Division, National Research Council, Ottawa, Canada. Issued as N.R.C. No. 2439.

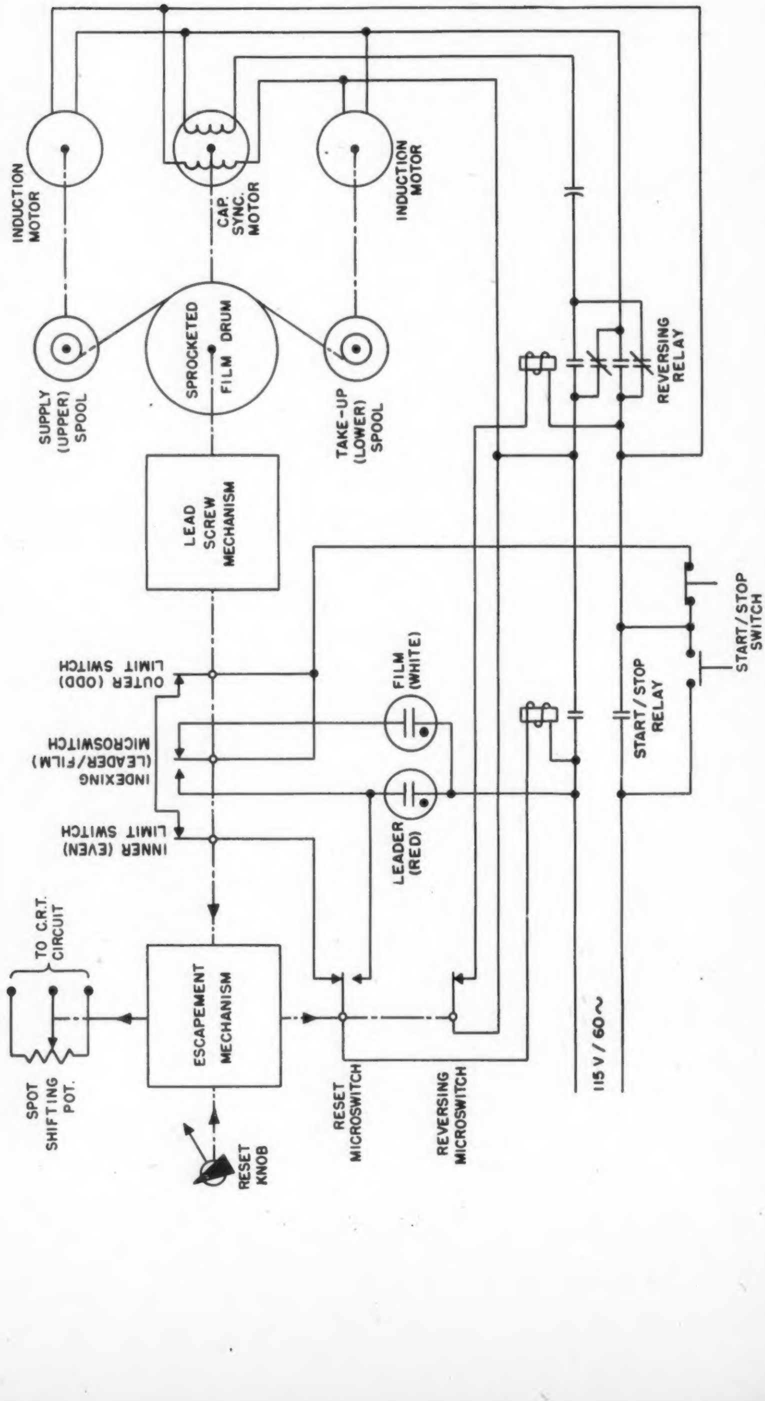
INDUCTION MOTOR
CAP. SYNC. MOTOR
INDUCTION MOTOR
SUPPLY (UPPER) SPOOL
SPROCKETED FILM DRUM
TAKE-UP (LOWER) SPOOL
REVERSING RELAY
LEAD SCREW MECHANISM
OUTER (ODD) LIMIT SWITCH
INDEXING (LEADER/FILM) MICROSWITCH
INNER (EVEN) LIMIT SWITCH
FILM (WHITE)
LEADER (RED)
START/STOP RELAY
START/STOP SWITCH
TO C.R.T. CIRCUIT
ESCAPEMENT MECHANISM
SPOT SHIFTING POT.
RESET KNOB
RESET MICROSWITCH
REVERSING MICROSWITCH
115 V / 60~

FIG. 1.

the remaining two could be eliminated if a double-pole, double-throw microswitch were available. The final arrangement is shown schematically in Fig. 1.

The film is drawn from a supply reel by a sprocketed drum of large diameter, driven by a synchronous motor through suitable reduction gearing. The film is exposed while on the drum and upon leaving the drum is wound on a take-up reel driven by an induction motor. The slip of the induction motor is used to take care of the variations in speed from empty to full spool conditions. Near the end of the film the drum motor is reversed and a second exposure is made while the film travels backwards. When rewinding, the functions of the supply and take-up reels are interchanged, thus it was necessary to provide each reel with a motor drive that may be switched accordingly.

The essential element is still the lead screw associated with the film drum, but now it actuates an escapement mechanism. At the end of each travel the escapement wheel moves one step, turning a potentiometer shaft about 15 degrees to deflect the spot one track separation. The wheel also serves as a cam to actuate a microswitch on odd steps and release it on even steps. This in turn operates the reversing relay controlling the direction of the drum motor and, at the same time, selects the proper take-up motor.

A second microswitch also associated with the escapement wheel functions only when a fresh film is loaded. Its purpose is to by-pass the inner limit switch after the escapement mechanism has been reset, and thus allow the drum motor to be started. When the leader has been run off the indexing microswitch stops the film and the camera waits for the reset knob to be released and the start button pushed.

The lead screw mechanism is provided with two limit switches, but only the inner one gets opened in normal circumstances and then only at the end of the last (20th) pass. The outer limit switch is purely insurance against failure of the reversing mechanism.

The operator has available two push buttons—start (black) and stop (red), two indicating lamps—white and red, and a resetting knob whose pointer serves to indicate, on a scale, the position of the escapement wheel. The white lamp is the one lit most of the time when the camera is running, the red lamp comes on in its place only at the very beginning and end of a film when the leader is being wound on or off the take-up spool.

The camera may be stopped and restarted at any time—but of course the usual precaution against fogging the film must be observed. Normal operating procedure is to load an unexposed film—which may be done in subdued light—winding a few inches of leader onto the take-up (lower) spool. Then with the camera closed, the reset knob is turned back to zero and held, and the start button is pushed momentarily. The camera will run only until all the leader is transferred to the take-up spool. The reset knob may then be released to position 1, and after any desired delay the start button may again be pushed to start the first exposure.

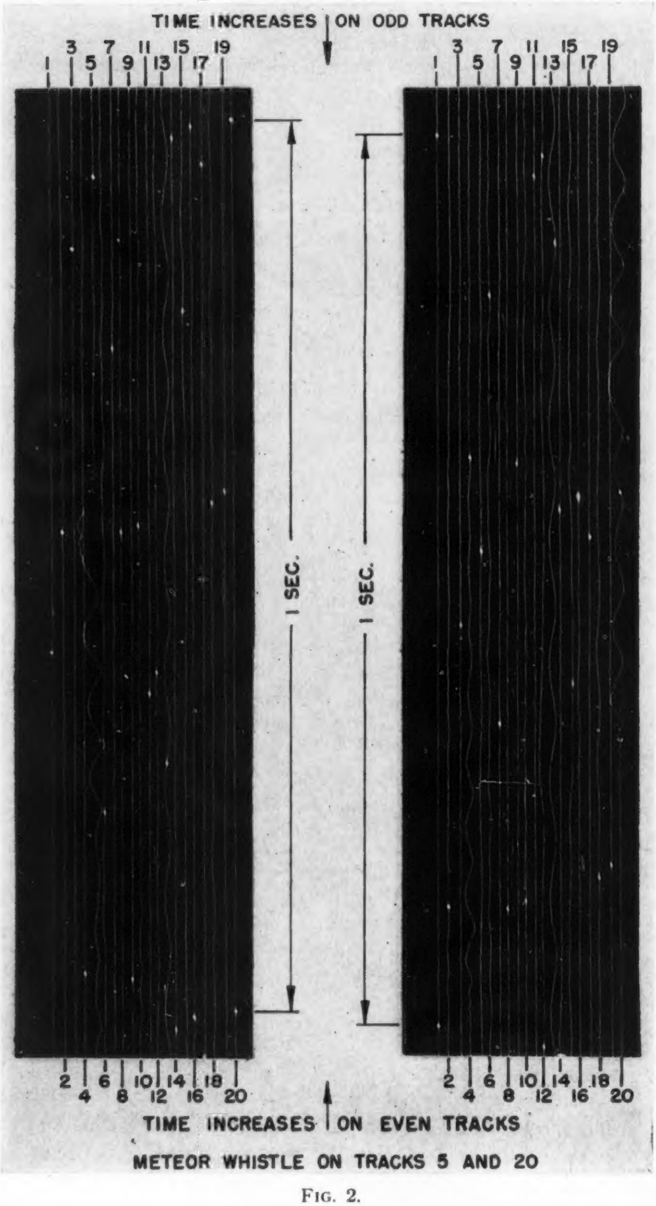
TIME INCREASES ON ODD TRACKS
1 3 5 7 9 11 13 15 17 19
1 3 5 7 9 11 13 15 17 19
1 SEC.
1 SEC.
2 4 6 8 10 12 14 16 18 20
2 4 6 8 10 12 14 16 18 20
TIME INCREASES ON EVEN TRACKS
METEOR WHISTLE ON TRACKS 5 AND 20

FIG. 2.

At the end of the last exposure the camera will continue to run past the normal reversing point and the indication will change from white to red. Only when all of the film and most of the leader is back in the initial loading position, will the inner limit switch stop the motors. The camera may now be safely opened and the film removed. It is necessary to avoid turning the sprocketed drum, by hand, more than a fraction of a turn when loading or unloading film. Otherwise damage to the lead screw and nut assembly may result.

The new driving motors and their control mechanisms have been added in such a manner that the present camera is a single compact unit. It has been in intermittent service for over a year and given no trouble.

Fig. 2 is an enlargement of two small portions of an experimental record taken for the purpose of determining meteor velocities by the doppler method. Here the desired events are random and not too frequent, with the result that most of the record is blank. It is in this type of service that the multiple use of film pays off.

The optical arrangement of the original camera as designed by Mr. R. D. Harrison of these laboratories has been retained, along with a portion of the cast aluminium body. The lens is a Zeiss Sonnar of aperture 1.5 and focal length 5 cm. which, when used with fast film such as Eastman Super XX, allows rapid writing speeds without excessive spot brightness.

INORGANIC NITROGEN IN PRECIPITATION AND ATMOSPHERIC SEDIMENTS[1]

By DeLoss H. Matheson[2]

Abstract

In an investigation covering 18 months, daily determinations were made of the inorganic nitrogen contained in precipitation and atmospheric sediments collected at Hamilton, Ont. The nitrogen fall for the whole period averaged 5.8 lb. N per acre per year. Sixty-one per cent of the total nitrogen was collected on 25% of the days when precipitation occurred. The balance, occurring on days without precipitation, is attributable solely to the sedimentation of dust. Ammonia nitrogen averaged 56% of the total, but the proportion for individual days varied widely.

Introduction

Analyses of rain and snow were started in January, 1949, to determine the extent to which precipitation might contribute significant amounts of inorganic nitrogen compounds to reservoirs and natural bodies of surface water. Although the greater proportion of the nitrogen input to these bodies comes from other sources, a study of the literature indicated that the amount contributed by precipitation might well be of significance when applied to water as it is when applied to land.

After some months of exploratory work it was found that appreciable amounts of these compounds were deposited on exposed surfaces in the absence of precipitation, owing to the sedimentation of dust. A regular sampling schedule was adopted and for 18 months, beginning July 1, 1949, samples were collected and analyzed, daily except Sundays and holidays.

Experimental

Samples were obtained by exposing an enamelled pail, 260 mm. in diameter (area 0.053 sq. m.) in a holder 75 cm. above a flat roof at the Hamilton Filtration Plant. Rain water collected was removed daily at noon, unless unavoidable circumstances prevented. If no precipitation had occurred or if only a small amount was present, the pail was rinsed several times with small portions of nitrogen-free distilled water, and the nitrogen determined in the washings. In a number of cases when storms occurred at convenient times, samples were removed from the pail at short intervals, to determine changes in concentration and in the rate of precipitation during the storm.

Ammonia was determined by distillation of an aliquot made alkaline with one drop of 25% nitrogen-free sodium hydroxide, nesslerization, and measurement by means of a filter photometer. Nitrate was determined in the residue remaining in the distilling flask by Devarda's method, the ammonia resulting

[1] *Manuscript received April 18, 1951.*
[2] *Chemist and Bacteriologist, Hamilton Filtration Plant, Hamilton, Ont.*

therefrom being distilled and determined as before. Nitrite was determined for a time by the sulphanilic acid – α-naphthylamine method, but as it was never found in more than insignificant traces this routine determination was discontinued.

Ammonia and nitrate were calculated to nitrogen. The concentration of nitrogen was determined when a sufficiently large sample of precipitation was available. Whether precipitation occurred or not, the nitrogen-fall for each day was calculated and expressed as milligrams nitrogen per square meter. When the collection period unavoidably extended over more than one day, the total nitrogen fall was computed and divided among the days as seemed reasonable, having regard to the prevailing meteorological conditions.

Total Nitrogen Fall

The total nitrogen fall during each month of the period under investigation is shown in Table I. Evidently the amount of inorganic nitrogen contributed to water bodies by precipitation is sufficient to be of biological significance. The monthly average of 52.5 mgm. per sq. m. is equivalent to 5.8 lb. per acre per year, an appreciable fraction of an average agricultural application to land. The ratio of ammonia to nitrate nitrogen is remarkably constant in the monthly totals, the former averaging 57% of the total nitrogen fall. The maximum nitrogen fall on one day, nearly 23 mgm. per sq. m., occurred on

TABLE I

TOTAL NITROGEN FALL, BY MONTHS

Month	Precipitation, in.	Total nitrogen fall, mgm. per sq. m.	Daily nitrogen fall, mgm. per sq. m.		
			Av.	Max.	Min.
1949					
July	2.60	55.6	1.85	15.70	0.33
Aug.	*	39.7	1.32	7.70	0.94
Sept.	1.35	43.2	1.43	5.64	0.46
Oct.	1.62	35.5	1.14	4.39	0.43
Nov.	2.11	46.0	1.53	11.65	0.35
Dec.	2.93	39.3	1.27	5.66	0.42
1950					
Jan.	3.66	43.5	1.40	5.08	0.33
Feb.	1.85	32.2	1.15	6.96	0.33
Mar.	1.58	35.5	1.19	5.10	0.37
Apr.	2.15	65.2	2.18	18.90	0.60
May	0.95	44.5	1.44	10.35	0.40
June	0.97	51.1	1.70	9.30	0.76
July	4.07	81.9	2.64	17.90	0.70
Aug.	4.35	87.0	2.80	22.95	0.35
Sept.	2.25	79.4	2.65	11.80	0.32
Oct.	2.36	60.9	1.96	8.30	0.43
Nov.	3.03	68.1	2.27	6.80	0.52
Dec.	1.11	41.9	1.34	8.50	0.48

**Data incomplete.*

Aug. 29, 1950, during a storm which precipitated 1.95 in. of rain. This nitrogen fall on one day was 30% of the month's total.

Nitrogen Fall on Days of No Precipitation

Appreciable amounts of inorganic nitrogen were collected on days of no precipitation, on the average about 40% of the month's total nitrogen fall. Table II shows these data by months. The daily average nitrogen fall for the whole period was 0.89 mgm. per sq. m. and the median was 0.85 mgm. per sq. m. The values for individual days ranged from 0.32 to 3.54 mgm. per sq. m.

TABLE II

NITROGEN FALL ON DAYS OF NO PRECIPITATION

Month	Number of days	Daily nitrogen fall, mgm. per sq. m.			Per cent of month's total nitrogen fall
		Av.	Max.	Min.	
1949					
July	22	0.57	1.60	0.33	24
Aug.	*	*	*	*	*
Sept.	21	0.71	0.77	0.46	35
Oct.	26	0.82	0.90	0.43	59
Nov.	23	0.73	1.25	0.35	37
Dec.	23	0.77	1.32	0.42	45
1950					
Jan.	16	0.58	0.90	0.33	21
Feb.	17	0.71	0.99	0.33	38
Mar.	23	0.75	1.36	0.37	49
Apr.	18	1.03	1.26	0.55	27
May	27	0.97	1.74	0.40	59
June	21	0.95	1.85	0.89	39
July	21	0.86	1.32	0.63	22
Aug.	22	0.98	1.27	0.35	26
Sept.	26	1.60	3.54	0.32	52
Oct.	26	1.19	1.57	0.43	51
Nov.	19	1.08	1.70	0.52	30
Dec.	26	0.85	1.57	0.48	53
Average		0.89			39

**Inadequate data.*

Ammonia nitrogen made up 60% of the total nitrogen fall on days of no precipitation. This proportion of ammonia nitrogen was not greatly different from that for the total nitrogen fall.

The nitrogen fall on precipitationless days was somewhat higher during the summer when, as might be expected, more dust is carried by the air. A detailed study of individual days failed to disclose any definite correlation between atmospheric conditions and the amount of nitrogen collected. Koch (4) thought that prolonged precipitation might wash the atmosphere free of dust and thus be followed by days of low nitrogen fall, but evidence of this was not found in this work. Also, collections obtained on days when the

country was covered by frozen snow were not always lower than the average days for the same month. The unusually heavy clouds occurring on Aug. 24, 1950, which were attributed to forest fires in Alberta did not cause any marked change in the nitrogen collected on this or the following days. Evidently the variations found in the day to day collections on precipitationless days are due to more complex changes in atmospheric conditions than can be determined by the qualitative observations made. However, a definite increase in nitrogen collected was noted on days when there was a trace of rain or a heavy dew; the moist surface of the receiver undoubtedly acting as a more efficient collector of dust.

Nitrogen Fall on Days of Precipitation

The nitrogen fall on days when precipitation occurred varied from less than 1.0 mgm. per sq. m. per day (compared with the average for no-precipitation

TABLE III

CONCENTRATION OF INORGANIC NITROGEN IN PRECIPITATION

Representative storms

Date 1950	Details	Precipitation, in.	Concentration, p.p.m. N		
			NH_3	NO_2	Total
Long precipitations					
Jan. 24	12 Hr. sleet	0.49	0.21	0.20	0.41
Mar. 23	Quiet wet snow	0.44	0.20	0.25	0.45
Mar. 27	10 Hr. rain	0.44	0.32	0.21	0.53
Apr. 4	12 Hr. rain	1.00	0.45	0.30	0.75
May 10	9 Hr. rain	0.23	0.20	0.43	0.63
May 19	15 Hr. rain	0.51	0.51	0.30	0.81
July 19–20	2 Day rain	1.01	0.12	0.18	0.30
Aug. 26	Quiet 9 hr. rain	0.13	0.78	1.04	1.82
Aug. 29	Heavy rain	1.95	0.27	0.19	0.46
Sept. 1	Slow drizzle	0.60	0.34	0.25	0.59
Sept. 11	15 Hr. rain	0.82	0.55	0.13	0.68
Oct. 10	Rain	0.76	0.24	0.25	0.49
Oct. 12	Rain	0.86	0.16	0.13	0.39
Oct. 28	Slow quiet rain	0.35	0.36	0.22	0.58
Short precipitations					
Apr. 10	Shower	0.09	0.50	0.42	0.92
July 14	Heavy shower	0.35	0.42	0.25	0.67
July 18	Light shower	0.22	0.42	0.28	0.70
July 25	2 Hr. heavy thunderstorm	0.45	0.30	0.23	0.53
July 29	10 Min. shower	0.05	0.73	0.22	0.95
Aug. 1	Intermittent thunderstorms all day	0.30	0.66	0.53	1.19
Aug. 2	Shower	0.11	0.69	0.81	1.50
Aug. 3	Shower	0.12	0.59	0.45	1.04
Aug. 31	Heavy 1 hr. storm	0.33	0.50	0.61	1.11

days of 0.89 mgm. per sq. m.) to a maximum of nearly 23 mgm. per sq. m., which occurred when a heavy thunderstorm precipitated 1.95 in. of rain. The next highest nitrogen fall on one day, 18.90 mgm. per sq. m. occurred in April 1950 when a 12 hr. quiet rain precipitated 1.00 in. of water. The median value for the nitrogen fall on days of precipitation falls within the bracket: 2.0–3.0 mgm. per sq. m. per day.

The concentration of inorganic nitrogen in precipitated water collected over a 24 hr. period varied widely. Data of representative precipitations are shown in Table III. Generally, short sharp rains yielded more concentrated solutions than did longer precipitations. The ratio of ammonia to nitrate nitrogen varied widely. Contrary to expectations, the nitrate was as often in excess of ammonia in the precipitation from quiet rains as it was in samples collected during electrical storms.

TABLE IV

CONCENTRATION OF INORGANIC NITROGEN IN PRECIPITATION

Illustrating changes during progress of representative storms

Date	Details	Precipitation, in.	Concentration, p.p.m. N	Nitrogen fall, mgm. per sq. m.
July 6 '49	A 3 hr. rain			
	1st 50 min.	0.09	1.00	4.75
	2nd 50 min.	0.46	0.48	5.60
	3rd 50 min.	0.44	0.76	8.60
	4th 50 min.	0.03	1.79	1.50
Feb. 14 '50	Snow and rain			
	First 12 hr.	0.41	0.21	0.99
	Last 3 hr.	0.23	0.14	0.66

A number of storms were sampled at shorter intervals to detect any change in concentration during the progress of the precipitation. Data of two representative storms are shown in Table IV. The effect of washing out the atmosphere by the first portion of the precipitation is not evident from these and other data.

Since prolonged precipitations are usually of low concentration while short precipitations are higher, the total nitrogen fall in one day of precipitation varies irregularly and over a very wide range. Prolonged precipitations lasting one or more days may result in a lower nitrogen fall per day than will be the case with shorter, more intense storms.

Discussion

Early analyses of rain water were published by R. A. Smith in 1872. F. W. Clarke (1) in 1920 summarized the literature to date, and quoted the data of authorities in widely separated parts of the world who reported nitrogen falls ranging from 0.60 to 9.20 lb. per acre per year. He also noted that in

most cases ammonia nitrogen exceeded nitrate nitrogen, but in the tropics the reverse seemed true. A large part of the inorganic nitrogen compounds in the air was considered to come from the soil, but some originated in electrical discharges.

Daniel *et al.* (2) in the United States found nitrate nitrogen in rain corresponding to 0.98 lb. per acre per year. Narayamaswami (5) at Bombay found 0.08 to 0.24 p.p.m. nitrate nitrogen in rain, the higher values occurring during thunderstorms. Ingham (3) in South Africa exposed dilute sulphuric acid in shallow trays and collected ammonia nitrogen equivalent to 6 to 45 lb. nitrogen per acre per year, the variation in values being attributed to variable winds. Ammonia was found to be absorbed on leaf surfaces to such an extent that rain dripping from leaves contained five times as much ammonia as rain collected in a rain gauge set in the open. Roelofsen (6) analyzed rain water collected in a gauge on the Deli river watershed. The collected water was accumulated for a year and analyzed once yearly for 15 years. The concentration of nitrogen averaged 1.5 p.p.m., varying from 0.82 to 2.18 p.p.m. In Ceylon, Koch (4) determined the nitrogen content of rain on semimonthly collections over a period of one year, and found a deposit of 7.5 lb. ammonia nitrogen and 5.4 lb. nitrate nitrogen per acre per year, with an annual precipitation of 118 in. The highest amounts were brought down by heavy rains following drought. The quantity found depended on total rainfall, intensity of thunderstorms, and the extent to which the atmosphere had been washed out by previous rains.

Shutt (7) reported the nitrogen content of rain and snow collected at Ottawa, Canada, in an investigation lasting over a period of 17 years. The average nitrogen deposit was 6.916 lb. per acre per year, varying from 4.3 to 11.5 lb. per acre. It was not possible to account satisfactorily for the year to year variations in the total deposit. Fifty-six per cent of the nitrogen was in the form of ammonia, 13% as albuminoid ammonia and 31% as nitrate and nitrite. The nitrogen fall per month, averaged over the whole period, varied from 0.34 lb. per acre in February to 0.85 lb. per acre in August, and was closely related to rainfall as its principal determinant. It was considered that the annual nitrogen fall was gradually increasing owing to an increase in the ammonia fraction which was attributed to the increasing use of bituminous coal in the near-by city.

In the period covered by this investigation the total nitrogen fall of 5.8 lb. per acre per year is consistent with the amounts previously reported in the literature, although significantly lower than that reported by Shutt (7). In this case, however, the nitrogen in albuminoid ammonia, which constituted 13% of Shutt's total, was not determined. More than one-third of the nitrogen recovered here was collected on days when no precipitation occurred, which suggests that at least a large portion of the nitrogen is associated with dust particles in the atmosphere. This may be accounted for in part by the fact that the collections were made at the edge of a highly industrialized city.

Summary

Significant amounts of inorganic nitrogen compounds are contributed to soil and the surface of water bodies by the deposit of precipitation and atmospheric sediments. In the last six months of 1949, 259 mgm. N per sq. m. were collected, equivalent to 5.2 lb. per acre per year; while in 1950 the collections totalled 691 mgm. per sq. m., or 6.2 lb. per acre per year.

Sixty-one per cent of the total nitrogen fall occurred on the 25% of the days when some precipitation occurred. On many of these days the precipitation was slight. The balance of the total nitrogen fall occurred on days of no precipitation, and accordingly is attributed solely to the sedimentation of dust.

The inorganic nitrogen precipitated during the whole period averaged 56% ammonia nitrogen. Individual samples varied widely from day to day, but the proportion of ammonia nitrogen did not vary significantly on days with or without precipitation.

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THE PRODUCTION OF 2,3-BUTANEDIOL FROM SULPHITE WASTE LIQUOR[1]

By D. Murphy[2] and D. W. Stranks[3]

Abstract

Pseudomonas hydrophila, *Aerobacter aerogenes*, and strains of *Serratia* grown on suitably treated sulphite waste liquor gave good yields of 2, 3-butanediol and ethanol in still- and shake-cultures. *Bacillus polymyxa* gave less satisfactory yields and *Bacillus subtilis* grew only when the medium was mixed with sugar-beet molasses. The method of treatment of the sulphite waste liquor was simple. After boiling and aeration the pH was set at 8.5 with ammonia or ammonium hydroxide, salts were added, and the medium autoclaved, during which the pH dropped to 5.7–6.4. In shake-cultures it was necessary to add calcium carbonate to control the pH during fermentation. Good yields of products were obtained when the organisms were grown on sulphite waste liquor concentrated to half volume before treatment. Doubling the sugar content of the medium by means of sugar-beet molasses also gave good growth and yields.

Introduction

In recent years much attention has been paid to the fermentative utilization of the sugars present in sulphite waste liquor. The two processes most studied have been the production of food yeast and of ethanol. The former became a large industry in Germany during the second World War and has been well surveyed by Skoog (7). Production of ethanol has also reached the industrial scale in several countries (3). The number of publications on other fermentations using sulphite waste liquor has been small. Leonard, Peterson, and Johnson (4) used *Lactobacillus pentosus* to produce lactic acid, and Wiley and his co-workers fermented sulphite waste liquor with *Clostridium butylicum* (Fitz) to obtain acetone and butanol (11).

No work has been reported in which sulphite waste liquor has been used for the fermentative production of 2, 3-butanediol, though Perlman reported satisfactory yields of this compound when he fermented wood hydrolyzates with *Aerobacter aerogenes* (6). As part of the program of research that has been carried on for several years in these laboratories, it was decided to investigate the production of butanediol by fermentation of sulphite waste liquor, using a variety of organisms.

Materials and Methods

The organisms used were *Bacillus polymyxa*, strains C2(1), C3(2), and C42(3)E13, local isolates, and strain UA206, received from the University of Alberta; *Aerobacter aerogenes* strain M148, isolated in these laboratories; two

[1] *Manuscript received February 19, 1951.*
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as Paper No. 120 on the Industrial Utilization of Wastes and Surpluses and as N.R.C. No. 2491.
[2] *Biochemist, Industrial Utilization Investigations.*
[3] *Bacteriologist, Industrial Utilization Investigations.*

strains of *Bacillus subtilis*, B44, originally No. S–8 Ford strain received from Dr. Gunsalus, Cornell University, and strain B200, a local isolate; two strains of *Serratia*, S4 which was originally A. T. C. C. No. 6065 (*S. anolium*) and S16 received from Dr. B. Eagles, University of British Columbia, as FV (*S. marcescens*); and two strains of *Pseudomonas hydrophila*, 491 and 492, both received from Dr. Reed, Queen's University, Kingston, Ont., and isolated from fish: these were originally received as *Proteus hydrophilis*. All the organisms were maintained on molasses agar slopes under mineral oil: no attempt was made to acclimatize them to the sulphite waste liquor.

Three samples of sulphite waste liquor were obtained, hereafter referred to as Liquors A, B, and C. Liquors A and C contained 2.6 to 3.0% copper-reducing substances. Liquor B contained 4.28%. About 75% of the reducing substances were fermentable in the three samples. It was necessary to treat the sulphite waste liquor before fermentation. The method used originally was based on that evolved by Leonard *et al.* (4). The sulphite waste liquor was boiled and aerated for 15 min. to remove sulphur dioxide, and when cool, the pH was brought to 8.5 with lime. After the liquor had stood from 30 min. to 12 hr. the pH was brought to 7.0 with carbon dioxide, and it was then filtered or centrifuged and used to prepare the medium. Setting the pH with carbon dioxide was troublesome because of frothing. By omitting this step and autoclaving the lime-free liquor the pH dropped to around 6.0. The pH of the liquor could also be set with sodium or potassium hydroxide or sodium carbonate. Ammonia gas or ammonium hydroxide proved the most satisfactory alkali to use. In the latter part of the work the treatment of the sulphite waste liquor was standardized to the following method: boiling and aerating for 15 min., cooling and adding nutrients, setting to pH 8.5 with ammonia, and making to the original volume. Sterilization at 15 p.s.i. for 10 min. caused the pH to fall to around 6.0. In a small number of runs (about 2% of the total) no growth could be obtained. This phenomenon was investigated but could not be satisfactorily explained.

In the preliminary work the following salts were added: dibasic ammonium phosphate 0.1%, ammonium sulphate 0.43%, potassium chloride 0.05%, and magnesium sulphate heptahydrate 0.04%. It was later found necessary to add only the ammonium phosphate. To provide growth-factors 5 ml. of 10% molasses was added to each 100 ml. of medium before sterilization.

Some of the work was done with sulphite waste liquor that had been concentrated to half its original volume. The liquor was concentrated either in an all glass evaporator, as described by Bartholomew (2), or by direct heat, the latter causing a certain destruction of carbohydrate. It was not difficult to concentrate Liquors A or C to less than half volume, but Liquor B, because of its higher solids content, could not be easily concentrated. As received, this sample approximated to concentrated Liquor A or C.

In all the experiments the amount of medium used was 100 ml., which was dispensed in 500 ml. Erlenmeyer flasks. Cotton plugs were used. The inocu-

lum was grown on a 5% molasses medium with 0.5% added cornsteep liquor for 20 hr.: 5 to 10 ml. of inoculum was used for each flask. With sulphite waste liquor it was necessary to use a heavy inoculum. In the preliminary experiments (still-cultures) the flasks were shaken for one minute every 12 hr. In the latter part of the work all the flasks were shaken continuously on a Gump rotating shaker at 100 r.p.m. The temperature of incubation was 35°C.

Reducing substances in the sulphite waste liquor were determined by the method of Somogyi and Shaffer, as modified by Underkofler *et al.* (10). Before hydrolysis the solution was cleared with zinc hydroxide (8, 9). Butanediol was determined by the butanol extraction and periodate oxidation method of Leslie and Castagne (5). In every case a blank, prepared from the treated sample of sulphite waste liquor with the appropriate amount of inoculum, was used. Ethanol was determined by distillation and dichromate oxidation.

Results

Still-cultures

Strains of *B. polymyxa*, *Pseudomonas hydrophila* and *Serratia* were tested for growth and butanediol production on Liquor A in still culture. The only salt added was dibasic ammonium phosphate. The results, given in Table I, show that the diol: ethanol ratios were low. This was to be expected, however, since the flasks were only occasionally shaken and hence the conditions were

TABLE I

PRODUCTION OF BUTANEDIOL AND ETHANOL BY STRAINS OF *Pseudomonas hydrophila*, *Serratia*, AND *Bacillus polymyxa* ON LIQUOR "A" MEDIUM IN STILL-CULTURE. TIME = 72 HR.

(Yields expressed as gm. per 100 ml.; 0.1% $(NH_4)_2HPO_4$ added)

Organism		Product	
		Butanediol	Ethanol
P. hydrophila	491	0.77	0.38
P. hydrophila	492	0.76	0.35
S. anolium	S4	0.68	0.27
S. marcescens	S16	0.54	0.27
B. polymyxa	C2(1)	0.49	—
B. polymyxa	UA206	0.32	—

nearly anaerobic. With *P. hydrophila* the fermentation went quickly and yields such as those shown could be obtained in 48 hr. Yields similar to those shown in this table were also obtained when sodium or potassium hydroxide or ammonia gas was used to set the pH. *Bacillus subtilis* could not be induced to grow well on the liquor, unless 3% molasses (1.5% sugar) was added to the medium. Adding organic supplements such as malt sprouts did not noticeably increase the yield from any of the organisms tested.

The various organisms were also tested for growth and butanediol production on Liquor A concentrated to half volume, and the results are set out in Table II. The strains of *P. hydrophila* gave yields approaching those that would be expected from a molasses medium of similar sugar content. *S. anolium* approached most closely to *P. hydrophila* in yields of products. *B. polymyxa* grew on this medium also, but in four days it did not produce as much diol as it produced on normal strength liquor. Attempts were made to clear the medium with aluminum sulphate but yields of butanediol were markedly decreased with increase of clearing agent. *P. hydrophila* also grew on liquors concentrated to 37.5% and 25% of the original volume but the yields of butanediol did not show a corresponding increase over those obtained with liquor concentrated to 50% of the original volume. The much higher solids contents of the media may have inhibited the growth of the organism.

TABLE II*

BUTANEDIOL AND ETHANOL PRODUCTION BY STRAINS OF *Bacillus polymyxa*, *Serratia*, AND *Pseudomonas hydrophila* ON MEDIA PREPARED FROM VARIOUS AMOUNTS OF LIQUOR "A" CONCENTRATED TO 50% ORIGINAL VOLUME

(Yields expressed as gm. per 100 ml. of medium)

Organism		Composition of medium	Diol	Ethanol
S. anolium	S4	50 ml. SWL + 50 ml. water	0.66	0.18
S. anolium	S4	100 ml. SWL	1.23	0.31
S. marcescens	S16	50 ml. SWL + 50 ml. water	0.60	0.18
S. marcescens	S16	100 ml. SWL	1.07	0.35
B. polymyxa	C3(2)	50 ml. SWL + 50 ml. water	0.66	0.37
B. polymyxa	C3(2)	75 ml. SWL + 25 ml. water	0.68	0.37
B. polymyxa	C3(2)	100 ml. SWL	0.68	0.38
B. polymyxa	C42(3)E13	50 ml. SWL + 50 ml. water	0.77	0.31
B. polymyxa	C42(3)E13	75 ml. SWL + 25 ml. water	0.74	0.37
B. polymyxa	C42(3)E13	100 ml. SWL	0.66	0.28
P. hydrophila	491	100 ml. SWL	1.58	0.47
P. hydrophila	492	100 ml. SWL	1.63	0.38

The various organisms were also tested for butanediol production on the sample of Liquor B. This contained 4.28% reducing substances and 14.8% solids. *B. polymyxa* could not be induced to grow on this sample with any treatment. *Serratia* grew fairly well on the unconcentrated liquor, giving 1.04% butanediol in 96 hr., but only gave 0.21% on the liquor concentrated to 50% original volume. *P. hydrophila* grew very strongly on all concentrations of the liquor and gave good yields of diol and ethanol (Table III). It will be noted that there was a falling off in the relative yield of diol as the concentration of the liquor was raised.

Shake-cultures

Shake-cultures have the advantage over still-cultures of giving a faster fermentation: the various organisms were therefore tested under these conditions. The greater part of this work was done with strains of *P. hydrophila* and *Aerobacter aerogenes*. *B. polymyxa* and *Serratia* did not show any im-

provement in yield over those obtained with still-cultures. No difficulty had been met in controlling the pH of the medium during fermentation in still-culture. When the sulphite waste medium was shaken in air, however, it rapidly became very acid (pH 4.5 or lower): 2% calcium carbonate added

TABLE III

BUTANEDIOL AND ETHANOL PRODUCTION BY *Pseudomonas hydrophila* STRAIN 492 GROWN ON DIFFERENT MIXTURES OF LIQUOR "B" CONCENTRATED TO HALF ORIGINAL VOLUME. TIME = 96 HR.

(Yields expressed as gm. per 100 ml. of medium)

Composition of medium		Diol	Ethanol
Concentrated sulphite waste liquor, ml.	Water ml.		
50	50	1.27	0.37
75	25	1.82	0.52
100	—	2.22	0.71

before or after sterilization kept the pH in the range 5.7 to 6.4 during fermentation in shake-flasks. *P. hydrophila* and *A. aerogenes* were compared for butanediol production on Liquor A set at various pH levels (Table IV). *P. hydrophila* grew on all samples while *A. aerogenes* grew on those set from pH 5.8 upwards. It is notable that *P. hydrophila* produced considerably more butanediol, though it was lower in ethanol.

A series of experiments was carried out in which the two organisms were compared for butanediol and ethanol yield and for sugar consumption on the

TABLE IV

BUTANEDIOL AND ETHANOL PRODUCTION BY *Pseudomonas hydrophila* 491 AND *Aerobacter aerogenes* M148 GROWN ON NORMAL STRENGTH LIQUOR "A" SET AT VARIOUS pH LEVELS WITH AMMONIUM HYDROXIDE AND AUTOCLAVED IN THE PRESENCE OF 2% CALCIUM CARBONATE. TIME = 72 HR.

(Yields expressed as gm. per 100 ml. of medium)

Organism	pH before sterilization	pH after sterilization	Yields	
			Diol	Ethanol
P. hydrophila 491	3.8	5.88	0.98	0.21
	4.8	6.02	1.03	0.21
	5.8	6.00	0.99	0.20
	6.67	6.11	1.01	0.21
	7.6	6.29	0.97	0.21
	8.5	7.18	0.96	0.23
A. aerogenes M148	3.8	5.88	—	—
	4.8	6.02	—	—
	5.8	6.00	0.80	0.32
	6.67	6.11	0.77	0.32
	7.6	6.29	0.80	0.30
	8.5	7.18	0.73	0.26

TABLE V

COMPARISON OF BUTANEDIOL AND ETHANOL PRODUCTION AND SUGAR CONSUMPTION BY *Pseudomonas hydrophila* 491 AND *Aerobacter aerogenes* M148 GROWN ON NORMAL STRENGTH AND CONCENTRATED LIQUOR "C" TREATED WITH AMMONIUM HYDROXIDE. TIME = 72 HR.

(Yields expressed as gm. per 100 ml. of medium)

Treatment of SWL	Yields		Initial sugar, %	Residual sugar, %	Sugar fermented, %	Gm. diol per gm. sugar fermented	Diol: ethanol ratio	Diol yield, % of theory
	Diol	Ethanol						
*Pseudomonas hydrophila**								
Normal SWL, pH 6.5	1.27	0.30	3.81	0.79	79.2	0.42	4.25	84.1
Normal SWL, pH 8.5	1.24	0.24	3.81	1.18	69.0	0.47	5.17	94.3
Concentrated SWL, pH 8.5	2.14	0.39	6.89	1.42	75.3	0.39	5.49	78.2
*Aerobacter aerogenes**								
Normal SWL, pH 6.5	1.02	0.31	3.80	1.10	71.1	0.33	3.29	75.6
Normal SWL, pH 8.5	0.90	0.33	3.80	1.15	69.7	0.34	2.77	67.9
Concentrated SWL, pH 8.5	1.85	0.54	6.27	2.20	63.3	0.41	3.44	91.0

**No growth occurred in the concentrated medium set at pH 6.5.*

various samples of sulphite waste liquor. Liquors A and C were used at both normal strength and after concentration to 50% volume; Liquor B was used at normal strength. Levels of pH chosen were 6.5 and 8.5 and the neutralizing agents were ammonium and sodium hydroxides. All results were similar and only one example need be given: this is shown in Table V, which compares the yields obtained on normal and concentrated Liquor C. It will be seen that, on the whole, *P. hydrophila* fermented more of the available reducing substances than *A. aerogenes* and that in the normal strength liquor *P. hydrophila* produced considerably more butanediol per gram of sugar fermented. The diol:ethanol ratios were smaller with *A. aerogenes* than with *P. hydrophila*.

On the basis of several such runs it was concluded that *P. hydrophila* gave a better fermentation on Liquors B and C, and that both organisms gave approximately equal results on Liquor A. The basis of judgement was the equation "grams of diol produced per gram of sugar fermented". The conclusion is that for each sample of sulphite waste liquor it will be necessary to determine the best concentration to be used, the optimum pH, and the organism best suited to the particular sample.

Several experiments were made in which the sugar concentration of the sulphite waste was doubled by adding 5% by weight of sugar-beet molasses. The result of a typical experiment using *A. aerogenes* is shown in Table VI. Fast fermentations and good yields were obtained with *P. hydrophila* and *A. aerogenes* on all samples of sulphite waste liquor tested in this way.

TABLE VI

BUTANEDIOL AND ETHANOL PRODUCTION AND SUGAR CONSUMPTION BY *Aerobacter aerogenes* M148* ON LIQUOR "A" WITH 5% ADDED SUGAR BEET MOLASSES. TIME = 72 HR.
(Yields expressed as gm. per 100 ml. of medium; initial sugar 4.66%)

Treatment of liquor	Yields		Residual sugar, %	Sugar fermented, %	Gm. diol per gm. sugar fermented	Diol yield, % of theoretical
	Diol	Ethanol				
Set pH 8.5, NaOH	1.63	0.56	0.88	81.1	0.43	86.3
Set pH 6.5, NH_4OH	1.66	0.63	0.88	81.1	0.43	87.8
Set pH 8.5, NH_4OH	1.64	0.62	0.88	81.1	0.43	86.8

**With this organism no growth occurred in medium set at pH 6.5 with sodium hydroxide*

Discussion

This work has shown that it is possible to ferment samples of sulphite waste liquor with various organisms and to obtain good yields of butanediol and ethanol. The method of treatment evolved is comparatively simple if ammonia gas is used for adjusting the pH. Adams and Leslie (1) preferred ammonia gas to calcium carbonate for controlling the pH during the fermentation of whole wheat mashes by *B. polymyxa* because of its inherent sterility, range of control, ease of addition, and lower cost. These considerations also apply in the preparation of sulphite waste liquor for this fermentation. The

method of treatment recommended by Leonard *et al.* (4) makes it necessary to use a filtration or centrifugation step in preparing the medium. The troublesome neutralization with carbon dioxide advocated by these workers has also been eliminated and the addition of salts to the medium has been kept at a minimum.

As far as can be ascertained this is the first fermentation that has been found to run consistently on sulphite waste liquor concentrated to half normal volume. Experiments using this strength of liquor have been carried out in a routine manner with no special precautions. Leonard *et al.* (4) in their study on the production of lactic acid by *Lactobacillus pentosus* found that fermentation was slow and irregular on concentrated liquor. They concluded that it would be impractical to use it. This was the only reference that could be found in the literature to the use of concentrated liquor for fermentation. Since the amount of sugar available in sulphite waste liquor is so small it would be of advantage if fermentations could be carried out in concentrated liquor.

Doubling the sugar content of the medium by adding beet molasses has much to recommend it. It gives a strong and active fermentation with yields of butanediol and ethanol approaching those obtained from a medium prepared entirely from molasses and containing the same amount of sugar. It also eliminates the necessity of concentrating the sulphite waste liquor when a medium with a relatively high sugar content is desired.

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